Aging Brain 3 (2023) 100059

Contents lists available at ScienceDirect

Aging Brain

journal homepage: www.elsevier.com/locate/nbas

Rostral anterior cingulate connectivity in older adults with subthreshold depressive symptoms: A preliminary study

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ARTICLE INFO

Article history:
Received 19 July 2022
Revised 7 December 2022
Accepted 7 December 2022
Available online 15 December 2022

Keywords:
Depression
Anterior Cingulate Cortex (ACC)
Aging
Functional Connectivity
Magnetic Resonance Imaging (MRI)
Subsyndromal depression

ABSTRACT

Subthreshold depressive symptoms are highly prevalent among older adults and are associated with numerous health risks including cognitive decline and decreased physical health. One brain region central to neuroanatomical models of depressive disorders is the anterior cingulate cortex (ACC). The rostral portion of the ACC—comprised of the pregenual ACC and subgenual ACC—is implicated in emotion control and reward processing. The goal of the current study was to examine how functional connectivity in subregions of the rostral ACC relate to depressive symptoms, measured by the Beck Depression Inventory-Second Edition, in an ethnically diverse sample of 28 community-dwelling older adults. Based on *meta*-analyses of previous studies in primarily young adults with clinical depression, we hypothesized that greater depressive symptoms would be associated with primarily increased resting-state functional connectivity from both the subgenual ACC and pregenual ACC to default mode network regions and the dorsolateral PFC. We instead found that higher depressive symptoms were associated with lower functional connectivity of the ACC to the dorsolateral PFC and regions within the default mode network, including from the subgenual ACC to the dorsolateral PFC and anterior cingulate and from the pregenual ACC to the middle cingulate gyrus. This preliminary study highlights brain alterations at subthreshold levels of depressive symptoms in older adults, which could serve as targets for interventions.
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Introduction

Millions of people worldwide suffer from functional impairment and decreased quality of life due to depressive symptomology, yet do not meet diagnostic criteria for major depressive disorder. Often referred to as subthreshold depression, these symptoms affect many more people than major depressive disorder, and are particularly prevalent in older adults [39]. In fact, subthreshold depression's prevalence rate is 31.1 % in adults over the age of 65, relative to 6.3 % for major depressive disorder [26]. This is

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https://doi.org/10.1016/j.nbas.2022.100059
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problematic as late-life subthreshold depression is associated with both adverse public health outcomes and negative individual outcomes, as longitudinal studies have shown increased healthcare utilization for older adults with subthreshold depression [5]. The individual negative health consequences of late-life subthreshold depression are significant and include cognitive deficits [19], decreased physical health [5], and increased suicidal ideation [11]. Moreover, a large *meta*-analysis of longitudinal cohort studies demonstrated that people with subthreshold depression have an increased risk of developing major depression [30]. The identification of neural mechanisms underlying subthreshold depression could help facilitate treatments and improve outcomes, particularly with respect to aging adults [55].

The anterior cingulate cortex (ACC) is central to neuroanatomical models of depressive disorders, including subthreshold depression. Morphological studies including *meta*-analyses show reduced volumes and thickness of the ACC in depression [7,23,51,58], and a large body of research employing resting-state functional magnetic resonance imaging (rsfMRI) has specifically implicated the rostral portion of the ACC in emotion control and reward processing (e.g., [9,14,50]. Ventromedial prefrontal regions such as the ACC are considered part of the default mode network (DMN), a network of interconnected structures, divided into the ventral medial prefrontal cortex (PFC), the dorsal medial prefrontal cortex; the posterior cingulate cortex and adjacent precuneus, and the lateral parietal cortex, that are suppressed during tasks that require external attention and active during relatively passive states [8,48]. Depression is characterized by altered resting-state functional connectivity (rsFC) within the DMN and from the DMN to the dorsolateral prefrontal cortex [21], portions of which are considered part of the DMN in updated models [8,36]. This is supported by recent *meta*-analyses showing that depression is associated decreased rsFC between the nucleus accumbens and the ventral medial PFC, and between DMN regions such as the posterior cingulate and the precuneus, as well as higher rsFC between the posterior cingulate, middle temporal gyrus and middle frontal gyrus [15,64]. In addition, the severity of depression was negatively correlated with rsFC between the posterior cingulate and middle frontal gyrus [64].

The rostral ACC is comprised of two subregions: the pregenual ACC (pACC) and the subgenual ACC (sACC), both of which are implicated in depression [13,59]. The pACC is a structurally and functionally heterogenous region that is broadly involved in cognitive processing and emotion regulation [41]. At rest, the pACC shows positive functional correlation with widespread brain regions that make up the DMN and affective network, with other subregions of the cingulate gyrus, and with brain regions involved in emotional processing such as the amygdala [62]. Resting-state functional activity of the pACC negatively correlates with resting-state activity in widespread areas of the sensorimotor network, cognitive network, and visual network [62]. The sACC is involved in modulating visceromotor function in response to emotional or sensory stimuli [46]. Resting-state functional connectivity studies show positive correlations between the sACC and regions including the medial PFC, orbitofrontal cortex, and temporal pole, and negative correlations with regions such as pre- and postcentral gyrus (i.e., sensorimotor network), dorsolateral PFC, and dorsolateral parietal cortex (i.e., cognitive network) [62]. Alterations in these connectivity patterns have been linked to clinical disorders, including depression.

Both the pACC and sACC show altered connectivity profiles in depression. The seminal rsFC study of depression found both the subgenual cingulate and the thalamus showed greater functional connectivity with the DMN in depressed individuals compared to controls, and within the depressed group, longer depressive episode length was correlated with functional connectivity in the subgenual cingulate [22]. A *meta*-analysis showed that one of the key features of major depressive disorder is increased functional connectivity between the DMN and regions within the subgenual PFC, including the sACC [24]. Similarly, there is evidence of increased rsFC between the pACC and dorsolateral PFC [13]. However, other studies show decreased resting-state connectivity of the rostral ACC to other brain regions in depression, such as a recent study that found decreased connectivity of the sACC to the dorsolateral PFC, insula, ventral temporal cortex, and parahippocampal gyrus in depressed patients compared to controls [10]. However, study samples in prior reports have been primarily comprised of younger adults, making the findings difficult to generalize to older adults given the widespread changes in ACC rsFC patterns with normal aging [9].

Concerning depression in older adults, the extant literature shows evidence of both increased and decreased connectivity from subregions of the ACC to the dorsolateral PFC and DMN regions. For example, one study showed that symptom severity in late-life depression is associated with increased functional connectivity between the dorsal ACC and the dorsolateral PFC [21]. Another study found increased positive correlation of the anterior dorsal ACC to the dorsolateral PFC and supplementary motor area, and a decreased correlation of the anterior dorsal ACC to regions including the orbitofrontal gyrus and anterior insula in older adults with subthreshold depression compared to controls [33]. Moreover, in a study of rsFC in late middle-aged (mean age 56 years) adults, Philippi and colleagues (2015) found higher levels of subthreshold depressive symptoms were associated with primarily enhanced resting-state functional correlation of subregions of the ACC with regions including the posterior cingulate, dorsolateral PFC, inferior frontal gyrus, parahippocampal gyrus, and the cerebellum. They also found depressive symptom severity was associated with reduced connectivity of the pACC with the striatum and reduced connectivity of the anterior sACC with the insula.

Given these heterogenous results, there is a clear need for investigation of how depressive symptoms relate to rostral ACC functional connectivity in older adulthood, particularly in persons with mild symptomology, who may be more responsive to lifestyle interventions rather than pharmacotherapy. Highlighting the clinical significance of this research focus, functional connectivity patterns have been shown to predict response to depression treatments including repetitive transcranial magnetic stimulation

(rTMS), psychotherapy, ketamine, and pharmacotherapy [1,18,28,40,47,54,60,61]. To address this knowledge gap, we examined the association between subthreshold depressive symptoms and rostral ACC rsFC in an older adult sample. Based on previous *meta*-analyses highlighting depression-related increases in functional connectivity within the DMN during activity and at rest [24,27,63], we hypothesized that greater depressive symptoms would be associated with primarily heightened functional connectivity from both the sACC and pACC to DMN regions.

Methods

Participants

Twenty-eight older adults between the ages of 60 and 83 were recruited from the metro Atlanta community as part of an exercise intervention study in which sedentary older adults were randomly assigned to either an aerobic or a balance and stretching intervention for 12 weeks, with imaging and questionnaire data collected before and after the intervention [38]. The current secondary data analyses focused on the baseline data from the intervention study. Inclusion criteria for the parent study included (1) having no prior diagnosis of major depressive disorder or neurological disease, including Alzheimer's disease, Parkinson's disease, or stroke; (2) being right-handed; and (3) being a native English speaker. Exclusion criteria included (1) any condition that would interfere with a magnetic resonance imaging (MRI) scan; (2) recent hospitalizations within the past 6 months; (3) untreated diabetes or hypertension; and (4) significant cognitive impairments, defined as a score on the Montreal Cognitive Assessment of < 24. Since participants were recruited for an exercise intervention study, they were also required to be sedentary (i.e., not engaging in structured physical exercise and/or not engaging in more than 30 min of moderate physical activity on four or more days of the week), to have a physician's approval for participation in an exercise study, and to be able to walk more than 400 m. This research was approved by the joint Institutional Review Board of the Atlanta Veteran's Affairs Medical Center and Emory University and was conducted in accordance with the Declaration of Helsinki. All participants provided informed consent. See Table 1 for a summary of participant demographic information.

Depressive symptom assessment

The severity of current depressive symptoms was assessed via the Beck Depression Inventory-II (BDI-II; [3]), a widely used, 21-item self-report measure that has been validated in older adults [49,52]. The BDI-II has high internal consistency in the general population of older adults (Cronbach's α = 0.86) [52]. This scale assesses the frequency and severity of depressive symptoms over the past two weeks, with a total score $\geq$ 14 suggestive of clinical depression [4]. A total of 8 participants met this criterion. Depressive symptoms were used as a continuous measure in all statistical analyses, with higher scores on the BDI-II indicating higher levels of depressive symptoms.

Table 1
Sample characteristics.

Age (years)	70.82 ± 5.80
Race (White/AA or Black)	15/13
Sex (% female)	71.43 %
BDI-II	8.68 ± 6.62

Note. BDI-II, Beck Depression Inventory-II; AA, African American

fMRI acquisition and pre-processing

The MRI scans were acquired on either a Siemens 3 T TIM Trio MRI scanner or a Siemens 3 T Prisma-Fit MRI (Erlangen, Germany). Previous analyses in the parent study ruled out scanner effects. High-resolution (1 mm^3) T1-weighted images were collected as an anatomical reference with the following parameters: 3D MPRAGE, TE = 3.02 ms, TR = 2600 ms; TI = 900 ms; FOV = 240 mm; FA = 8○; matrix size = 256 × 256, 176 × 1.0 mm sagittal slices. During the resting-state scan, the participants were instructed to look at a white fixation cross on a black background, to stay awake, and refrain from moving. The rsfMRI time course was acquired with a single shot gradient recalled echo planar imaging (*EPI*) sequence using the following parameters: FoV = 220 mm × 220 mm, matrix = 74 × 74, 48 slices, slice thickness = 3 mm, TR = 3000 ms, TE = 24 ms, FA = 90○, 192 measurements, acquisition time = 9:36 min.

The MR images were processed using AFNI [12] and FSL [53] software packages, as well as in-house Matlab scripts. The rsfMRI time course was corrected for slice-timing and bulk-head motion using a rigid-body transform to the first resting-state image. We used an ICA denoising procedure with FSL's Melodic (v3.04) and FIX (v1.066) using hand selected classifiers. This proceeded as follows. We selected 12 participants' resting state *EPI* runs at random and used Melodic's ICA classification on these timeseries. Melodic performed a high-pass filter of 100 s, bulk motion correction using MCFLIRT, spatial smoothing of 5 mm and single-session ICA with automatic dimensionality estimation. Output components were evaluated visually for each dataset in FSLeyes by a trained neuroimaging analyst with a *t*-threshold of +/- 2. Using information from the spatial distribution of suprathreshold voxel clusters, the average timeseries and power spectra, components were delineated as either artifact (movement, cardiac, respiratory, hardware) or signal (BOLD). Components with an apparent mix of noise and signal were referred to another neuroimaging analyst for consensus. Using FSL's FIX, we created a new classifier set using the designated components from the above process and performed regression filtering on all participants *EPI* timeseries using a threshold of 18 classifiers (based on a previous comparison of output from 15, 18 or 20 classifiers). Ventricles were then masked to further reduce influence of cerebrospinal fluid pulsatility. Images were spatially normalized to the Montreal Neurological Institute's (MNI) template using non-linear transforms [20]. Frame-to-frame displacement was calculated to censor the rsfMRI time series at a 0.5 mm threshold [45]. Finally, bandpass filter was applied

to the rsfMRI time series using a Chebyshev II filter at 0.01 to 0.1 Hz [29] and spatially smoothed with a 5 mm full-width-half-maximum Gaussian filter.

Seed-based rsfMRI analysis

Based on *a priori* hypotheses, we seeded from the bilateral pACC (MNI coordinates; x = -2, y = 47, z = -4; Fig. 1) and from bilateral sACC (x = -1, y = 24, z = -10; Fig. 1). A 5 mm radius sphere was used to extract an average seed time course that was cross-correlated with the time courses of all other voxels in the brain. Next, a Fisher z-transform was applied to all the cross-correlation values to normalize the distribution. This resulted in z-score maps, which are referred to as Z(CC). The Z(CC) maps are a single 3D volume per participant that can be related with depression scores to generate voxel-wise brain-behavior maps.

Statistical analyses

We conducted linear regression analyses to examine the relationships between BDI-II scores and the bilateral pACC and sACC seeded Z(CC) maps. All participants' Z(CC) maps were concatenated into a single file for either pACC or sACC. Then a linear regression for each voxel x,y,z was performed between Z(CC) and BDI-II in Matlab 2018b (Mathworks, Danvers, MA) via the inbuilt Levenberg-Marquardt nonlinear least squares algorithm nlinfit. To correct for multiple comparisons, a family-wise error (FWE) correction was performed at the cluster level on the resulting brain-behavior maps. Cluster correction was performed with AFNI's 3dClustSim using the mixed-model autocorrelation function option (-acf). Second-level regression maps were threshold at an uncorrected, lenient voxel threshold of $p < 0.05$. A minimum size of 39 contiguous voxels was calculated by the 3dClustSim algorithm to represent the minimal size required to assume family-wise error corrected whole brain (total N of voxels tested: 919 and 1,295 for sACC and pACC, respectively) significance. Following the FWE correction, clusters composed of 39 or more contiguous (nearest neighbor) voxels were considered significant. Additionally, regression graphs were created to further examine the relationship between the bilateral pACC and sACC seeded functional connectivity to the DMN. Statistical comparisons were computed using AFNI [12], JMP (SAS Institute, Cary, NC) and SPSS X (IBM Corp., Armonk, NY).

Results

Results are summarized in Table 2, Fig. 1, and Fig. 2. We found that negative correlations of the bilateral sACC with the right anterior cingulate and the bilateral middle frontal gyrus were stronger as a function of higher BDI-II scores. Similarly, we found an association of higher depressive symptoms with negative correlations between the bilateral pACC and a DMN region, the right middle cingulate cortex, as well as the left superior frontal gyrus and the thalamus.

Discussion

In this study, we examined how functional connectivity in subregions of the rostral ACC relate to subthreshold depressive symptoms in older adults. We expected to find increased functional connectivity of the sACC and pACC to DMN regions as a function of higher depressive symptom severity, based on previous literature, including a *meta*-analysis, showing heightened connectivity in these regions in depressed compared to non-depressed individuals [24,27,63]. Contrary to our hypothesis, we found subthreshold depressive symptoms were associated with only reduced functional connectivity of both the pACC and sACC to regions within the DMN, including the anterior and middle cingulate and a portion of the middle cingulate corresponding to the dorsolateral PFC, which is included in updated models of the DMN [8,36].

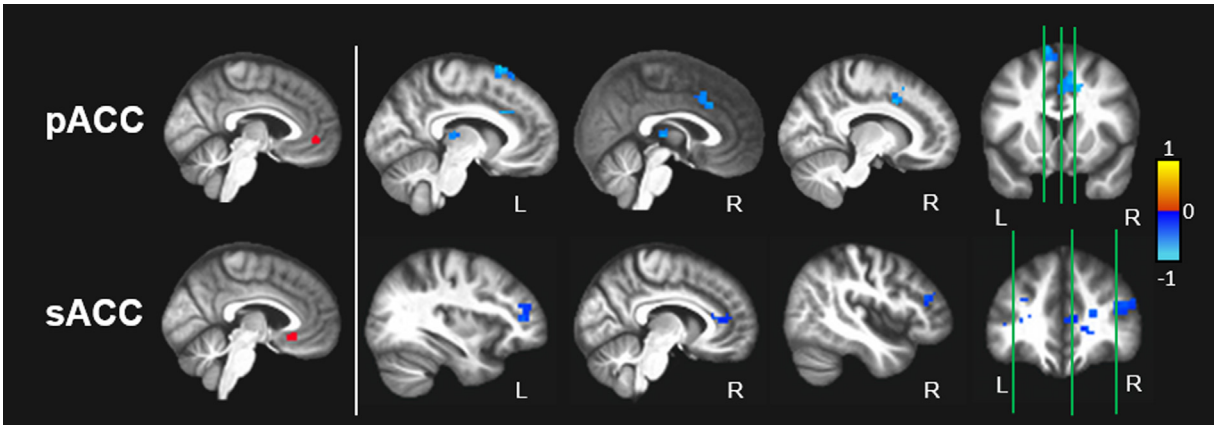


Fig. 1. Linear regressions of resting-state functional connectivity between pregenual and subgenual ACC seeds and BDI score. The brains to the left of the white line show the placement of the pACC and sACC seeds. The brains to the right of the white line show regions with negative (blue) correlations with BDI total scores. Statistical maps were thresholded at $p < 0.05$ FWER corrected, clusterized at 39 voxels, and overlayed on averaged MNI transformed T1-weighted images from participants in analysis. L = left, R = right. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Significant correlations between depressive symptoms and rsFC.

Brain Region	Side	Coordinates (Peak) X	y	z	Voxel Cluster Size
Subgenual ACC					
Anterior cingulate	R	15	27	18	112
Middle frontal gyrus	L	−33	45	12	90
	R	45	38	25	43
Pregenual ACC					
Middle cingulate cortex	R	14	8	38	94
Superior frontal gyrus	L	−8	18	72	53
Thalamus	M	0	−20	6	49

Note. All correlations were negative. Brain-behavior regression maps were thresholded at $p < 0.05$ FWER corrected and clusterized at 39 voxels. Coordinates are in MNI space with LPI orientation. Abbreviations: rsFC, resting-state functional connectivity; ACC, anterior cingulate cortex; L, left; R, right; M, midline.

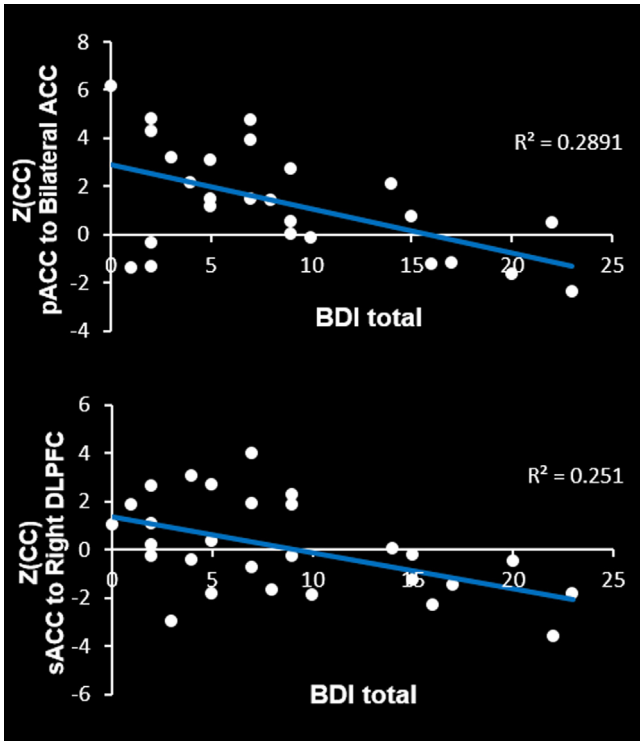


Fig. 2. The scatterplots show the z-transformed cross-correlations between resting state activity in seed regions and specific clusters of interest and their associations with depressive symptoms. The middle frontal gyrus region that was found to be significant includes the DLPFC. Abbreviations: pACC, pregenual anterior cingulate cortex; sACC, subgenual anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; BDI, Beck Depression Inventory.

We found that higher depressive symptoms were associated with a decrease in functional connectivity between sACC and the dorsolateral PFC. A number of previous studies, including in late-life clinical and subthreshold depression, have found increased ACC connectivity to the dorsolateral PFC related to depression [21,33], but decreased connectivity has also been found [44]. As a region of the executive control network, the dorsolateral PFC is involved in broad executive processes, including affect regulation, working memory, and problem solving. The reduced functional connectivity between the dorsolateral PFC and sACC may be indicative of an sACC that is unchecked without the dorsolateral PFC downregulating its activity. Hyperactivity of the sACC is associated with behavioral withdrawal and sad mood [17,24]. The sACC has also been a frequent target for deep brain stimulation in treatment resistant depression [37]. These studies have shown that targeted stimulation of sACC can reverse depressive symptoms. These findings suggest results from functional connectivity studies could have implications for depression treatment, and our results suggest that this may also be true for older adults.

The finding for the middle cingulate cortex is also worth noting. In healthy adults, the pACC demonstrates functional connectivity to all other areas of the cingulate gyrus, including the middle cingulate [62]. Thus, the negative correlation between depressive symptoms and functional connectivity from pACC to middle cingulate cortex observed in this study may represent a dysfunctional pattern of connectivity. Based on cytoarchitecture, receptor

mapping, and connections, the middle cingulate cortex has been distinguished from the adjacent dorsal ACC [42]. It is a complicated and heterogeneous brain region involved in processing pain, fear, and cognitive and motor tasks, among other functions [57]. The disconnection between pACC and middle cingulate cortex may partially account for some of the affective, somatic, and cognitive symptoms that characterize depression.

The literature in major depression, primarily comprising young adult samples, generally shows increased functional connectivity within the DMN and from DMN regions such as the ACC to other parts of the brain. Similarly, a study of late middle-aged adults found higher severity of subthreshold depressive symptoms was associated with mostly increased functional connectivity of rostral ACC subregions to the posterior cingulate, dorsolateral PFC, and other structures [44]. Previous *meta*-analyses also highlight depression-related increases in functional connectivity within the DMN during activity and at rest [24,27,63]. Nonetheless, findings in the literature are mixed, with some studies finding depression-related increases and others finding depression-related decreases in functional connectivity [24,27,56,63]. Another recent *meta*-analysis [56] found that depression was associated with reduced functional connectivity within a midline DMN core (posterior cingulate cortex and anterior medial PFC), thought to be involved in introspection, that integrates the function of the other subsystems. The *meta*-analysis did not find significant effects in two other DMN subsystems: A dorsal medial PFC subsystem (dorsal medial PFC, temporoparietal junction, lateral temporal cortex, and temporal pole) involved in mentalizing and metacognition and a medial temporal subsystem (ventral medial PFC, posterior inferior parietal lobule, retrosplenial cortex, parahippocampal cortex and hippocampal formation) involved in episodic decision-making about the future [2].

A previous study by Li and colleagues [33] most closely mirrors the current study, in that they examined rsFC related to subthreshold depression in older adults, seeding from the ACC. They found both increased and decreased rsFC of the ACC to the DMN and other regions in their subthreshold depression group compared to a control group. Most relevant to the present results, they observed decreased connectivity of the ACC to regions including the dorsolateral PFC, the thalamus, and other areas of the ACC in subthreshold depression, consistent with our findings. However, they also found subthreshold was associated with increased connectivity of the ACC to the dorsolateral PFC. After correction for multiple comparisons, we did not observe any relationships between depressive symptoms and increased functional connectivity of the ACC. The reasons for this inconsistency with previous literature and with our hypotheses are unclear, but could be due to power given our relatively small sample size. The characteristics of our sample may also be a factor, as we had a more ethnically diverse sample than other studies, with nearly 50 % Black/African-American participants, and participants were required to be sedentary since the parent study involved an exercise intervention. Future larger studies in diverse samples will help to clarify heterogenous findings in the literature.

Given the heterogeneity of symptom profiles in depression and evidence that different symptom clusters have distinct neurobiological correlates [16,31,35], it is possible that the combination of increased and decreased functional connectivity in previous studies reflects different aspects of the disorder. In line with this hypothesis, a large multi-site study identified four 'biotypes' of depression based on functional connectivity patterns, each of which corresponded to specific depressive symptom profiles [18]. For example, reduced connectivity in the ACC and orbitofrontal regions supporting motivation and incentive-salience evaluation characterized some of the biotypes, which were associated with anergia and fatigue. Increased connectivity in thalamic and frontostriatal networks that support reward processing, adaptive motor control, and action initiation were found in other biotypes and were associated with anhedonia and psychomotor retardation. Importantly, the four biotypes were associated with differential responses to treatment with rTMS to the dorsomedial PFC.

Other studies have also shown that functional connectivity patterns are linked to outcomes of depression treatments including psychotherapy, ketamine, and antidepressant medication [28,40,47,60,61], and to depression recurrence [32,60]. For example, one study reported that higher rsFC between sACC and middle temporal gyrus predicted a better response to nine weeks of psychotherapy for depression [54]. In another study, pharmacotherapy led to increased functional connectivity between the ACC and the posterior cingulate in depressed older adults after 12 weeks of treatment [1].

These findings point to the clinical significance of research that clarifies functional connectivity alterations in depressive disorders. Our preliminary study adds to this literature by demonstrating similar patterns of altered connectivity in older adults with subthreshold depressive symptoms. Most studies of functional connectivity in subthreshold depression focus on participants with scores in the clinical range on depression questionnaires, but who do not meet diagnostic criteria for major depression (e.g., [25,43]. The current study is unique in its demonstration of altered rostral ACC connectivity as a function of depressive symptom severity in older adults who had no diagnosis of major depression and mostly scored below the clinical cutoff on the BDI-II, showing the significance of even low levels of depressive symptoms in older adults. Importantly, our sample is unique in its inclusion of nearly equal numbers of Black and white participants, adding to the limited but much-needed neuroscience literature in diverse samples.

Our study had some notable limitations. Our sample size is relatively small and comprised primarily of female participants. As a result, results of this preliminary study should be interpreted cautiously. Future studies should recruit larger older adult samples of both men and women. Like most rsFC studies, our study does not include parallel electroencephalogram, which could be used to control for fluctuations in vigilance during the resting state scan [34]. In addition, though our rsfMRI protocol showed consistent signal after inspection during ICA, the scan duration (9:36) may not have been optimal for resting state stability

[6]. Finally, we only examined BDI-II total scores, which could mask differences related to different depressive symptom dimensions. Future studies should examine individual symptom dimensions (e.g., somatic symptoms, depressed mood, well-being) or symptom profiles.

In summary, the results show several alterations in the pattern of ACC connectivity in older adults with subthreshold depressive symptoms, extending previous findings in primarily young adults with major depression. This is important because depressive symptoms, even at subthreshold levels, exacerbate age-related brain changes. Therefore, it is crucial to understand the brain correlates of subthreshold depression in older adults, as this can help researchers identify brain regions to target with their interventions. Additional research is warranted that not only focuses on traditional depression treatments, but also on how positive modifiers of brain health (e.g., exercise, sleep, diet) can alter ACC functional connectivity. These studies should also consider how different symptom dimensions of depression relate to patterns of connectivity.

Funding

The views expressed in this work do not necessarily reflect those of the Department of Veterans Affairs or the United States Government. This work was supported by VA research awards: IK2RX000956 (KMM); I01RX002825, IK2RX000744 (JRN); I50RX002358 (CVNR- JRN & KMM). AMG is supported by the Georgia State University Brains & Behavior graduate student fellowship. VMD is supported by HRD #2112455 and R01 AG054046-04. LCK received funding from the Veterans Affairs Rehabilitation Research & Development Service (USA) grant IK1 RX002629. The funding sources had no role in the study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

CRediT authorship contribution statement

Andrew M. Gradone: Conceptualization, Methodology, Formal analysis, Writing – original draft, Visualization. **Gabriell Champion:** Formal analysis, Validation, Writing – original draft, Visualization. **Keith M. McGregor:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Writing – review & editing, Supervision, Funding acquisition. **Joe R. Nocera:** Investigation, Resources, Writing – review & editing, Funding acquisition. **Sarah J. Barber:** Writing – review & editing. **Lisa C. Krishnamurthy:** Software, Formal analysis, Writing – review & editing. **Vonetta M. Dotson:** Conceptualization, Methodology, Writing – original draft, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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